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Slow wilting components in pigeonpea (*Cajanus cajan*(L.) Millsp.)

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Abstract Partial resistance to Fusarium wilt was characterized based on root inoculation on seven pigeonpea genotypes with a virulent isolate of *Fusarium udum.* Resistance to wilt seems to be mainly due to localization of the pathogen in roots and vascular systems in the stem. Three components of resistance (wilting rate or incubation period, weighted wilt index and number of colonized plants) were determined and compared to those of susceptible genotypes Bahar and TTB7. There were significant differences between the genotypes for the resistance components. The mechanisms of resistance in the genotypes appeared to be different, with genotype ICP8863, having a longer incubation period, minimum wilt index and minimum pathogen colonization as compared to other resistant genotypes (ICP9174, ICP87119 and ICP8858). Wilting rate or incubation period and number of colonized plant were significantly correlated with resistance in adult plants in the field (AUDPC). Wilt index was useful in discriminating between genotypes that had a similar incubation period and number of colonized plants. The partial character of resistance is probably based on quantitative differences in localization capacity among the genotypes. A quantitative relationship between components, incubation period and number of colonized plants and the AUDPC, if verified for a large number of genotypes, may be used to obtain an index of resistance that may predict resistance levels in the field.

Keywords *Cajanus cajan* · Fusarium wilt · Components of wilt resistance

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Introduction

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is an important crop in semi-arid tropical and subtropical farming systems, providing quality vegetable protein, animal feed, and firewood. Wilt (*Fusarium udum* Butler) is a major biotic stress in almost all pigeonpea-growing states of India (Carlos Popelka et al. 2004; Ahlawat et al. 2005). Yield loss due to wilt has been reported to be over 50% (Marley and Hillocks 1996) or even up to 100% loss in grain yield (Okiror 2002b). Resistance or tolerance is vital to the management of wilt. However, vascular infection as well as the soil-borne nature of the disease complicates resistance evaluation. Standard methods for quick evaluation of resistance, optimal conditions for infection and inoculum potential of soil are questionable issues. Moreover, information on mechanism and genetics of

resistance is limited and largely contradictory (Jain and Reddy 1995; Reddy and Raju 1997) and, therefore, limits the production of resistant plants through selection based on morphological markers (Okiror 2002a). Lack of knowledge in resistance combined with the difficulties in working with a soil borne disease has impeded progress in developing resistant germplasm and cultivars. Phenotyping based on wilt incidence in the artificially root-inoculated plant or in sick plot trials takes a long time for symptom expression. Resistance components reflect underlying histological and physiological role on resistance mechanisms and they may be used as early selection criteria in resistance breeding (Baayen and van der Plas 1992). Resistance components against the important vascular wilt in carnation has been studied by Baayen et al.(1991) and three resistance components, localization ability, latent period and wilting rate have been evaluated as early selection criteria (Baayen and van der Plas 1992; Baayen 2005). The prospect of increasing the level of partial resistance by combining apparently different mechanisms of resistance to provide a more stable protection against a pathogen seems attractive as a breeding strategy (Parlevliet and Kuiper 1985). In the present communication, resistance components of vascular wilt in pigeonpea have been studied that may provide a basis of early selection of breeding materials.

Materials and methods

Plant material

Seeds of seven pigeonpea genotypes (ICP7035, ICP 87119, ICP 8863, ICP9174, ICP8858, Bahar and TTB7) were collected, particulars as given in Table 1. Seeds were surface sterilized with 1% sodium hypochlorite for 1 min and sown in cemented pots (18"×18"×17") filled with sterilized river bed sand—soil mixture in a 1:1 ratio and grown under glasshouse conditions.

Preparation of fungal culture

A highly pathogenic isolate AF13 (Sinha et al. 2008) of *Fusarium udum* was grown in potato-dextrose-agar plates and slants for 6 days at 28±1°C.

Root inoculation

For root inoculation a modified inoculation method (Mishra and Dhar 2005) was followed. Inoculation was done by pouring 3 ml suspension (1.2×10^6 conidia ml^{-1}) per plant on the soil after four weeks of sowing. Seven pots, each containing 10 seedlings of a single genotype constituted a replicate in a randomized complete block design with five replicates. For testing colonization, three similar sets of pots were maintained.

Field experiment for wilt observation in adult plants

Seven pigeonpea genotypes were sown in experimental sick plot of CSAUA&T Kanpur, which is a hot spot for the disease. Before sowing, plots were enriched with soil application of *F. udum* inoculum. Wilt observations were taken until the maturity of the plants. Area under disease progress curve (AUDPC, Campbell and Madden 1990) was calculated using the following formulae:

$$\mathrm{AUDPC} = \sum_{i=1}^{k} d(S_i + S_{i-1})/2$$

Table 1 Particulars of pigeonpea genotypes considered for inheritance of wilt resistance

Name	Genotype features	Source	Remarks
Bahar	Selection from local land race from Motihari, Bihar, India	IIPR, Kanpur	Susceptible
TTB7	Selection from landrace India	UAS, Bangalore	Susceptible
ICP7035	Selection from landrace India	IIPR, Kanpur	Moderately resistant
ICP 8863	Selection from ICP 7626 (P-15-3-3), a land race from Uttar Pradesh, India	ICRISAT, Hyderabad	Resistant
ICP 9174	Traditional cultivar/landrace from Kenya	ICRISAT, Hyderabad	Resistant
ICP 87119	Bulk pedigree selection from a cross ICP 1-6-W3 X ICP 1-6-W1 X C11	ICRISAT, Hyderabad	Resistant
ICP 8858	Selection from land race central India	IIPR, Kanpur	Resistant

where Si denotes disease severity at the end of week i, k is the number of successive evaluations of disease and 'd' is the interval between two evaluations.

Observations on components of quantitative resistance in pigeonpea

Wilt onset and progress were monitored at weekly interval on plants showing characteristic epinasty, yellowing and drooping symptoms. Incubation period, weighted wilt index and colonization of pathogen were observed in root inoculated and sick-plot infected plants.

Incubation period (IP)

IP was determined as days from inoculation to first wilt symptoms development.

Weighted wilt index (WI)

All plants were assessed externally as well as internally at 120 days after sowing (DAS) following a modified 0–5 scale (Baayen and van der Plas 1992): 0 no symptoms; 1 only internal discolouration limited to basal parts; 2 external symptoms of yellowing, epinasty and drooping of few leaves in one or two branches; 3 wilting symptoms in many branches; 4 wilting symptoms in whole plant and 5 death of whole plants. For WI estimation infection types were ranked according to their relative frequencies (Steffenson et al. 1985). The most prevalent infection was assigned to the highest rank and the least prevalent the lowest rank value and wilt weighted index (WI) was calculated for each replication and genotypes as follows:

$$\mathrm{WI} = \left[\sum (\text{rank} \times \text{infection type code})\right] \Big/ \sum \text{rank}.$$

Localization of the pathogen

Localization was measured by the number of plants colonized by the pathogen in the colonization test (Baayen and van der Plas 1992). Ten plants from each genotype were selected randomly and assessed at 15, 30 and 45 days after inoculation (DAI). For the sick plot experiment, plants were taken at 120 DAS. Basal stem pieces (2–4 mm) were taken for the colonization test and pieces were sterilized in ethanol and after flaming aseptically placed on PDA (Hi Media) plates containing 0.2% streptomycin and incubated for one week at 28°±1°C to allow the growth of *F. udum*.

Data analysis

Data for each component of quantitative resistance were analyzed using analysis of variance and the least significant difference was used to compare differences between the genotypes. A multiple regression analysis of the different components (as independent variables) and the area under disease progress curve (AUDPC, as response variable) of these genotypes was used to determine components that may be useful in predicting the performance of genotypes in the field.

Results

Components of quantitative resistance in root inoculation experiment

Susceptible genotypes had lower IP (28–31 days) than the IP (56–59) in resistant genotypes (Table 2). There were significant differences between the IP of susceptible genotypes (Bahar and TTB7) and resistant genotypes, and ICP8863 had the longest IP 85 days. Wilt index recorded in susceptible and resistant genotypes did not show significant variation except for ICP87119 and ICP8863, where a comparatively lower wilt index 0.66–0.80 was noted. Number of plants colonized at 15–30 DAI was low in susceptible genotypes along with moderately resistant ICP7035, whereas in resistant genotypes little or no colonization was recorded. However, colonization increased at 45 DAI as the fungus had been isolated from 21–24 plants of susceptible genotypes; the moderately resistant ICP7035 did not show an increase in number of colonized plants. Number of colonized plants (2–7) did not vary among the resistant genotypes and only in one plant of ICP8863 was colonization observed. Number of plants colonized, IP and WI did not separate moderately resistant ICP7035 from resistant genotypes except for ICP8863. Number of plants colonized and IP corresponded well with adult plant resistance

Table 2 Incubation period (IP), weighted wilt index (WI) and number of plants colonized after root inoculation on seven pigeonpea genotypes with *F. udum*

Genotypes	IP	WI at 90 DAI	Number of plants colonized at DAI		
			15	30	45
Bahar	28a[a]	1.56a	6a	8a	21a
TTB7	31a	1.66a	4a	5a	24a
ICP7035	56b	1.33b	1b	7a	7b
ICP 9174	61b	1.40b	0b	0b	2c
ICP 87119	56b	0.80c	0b	2b	5bc
ICP 8858	59b	1.66a	0b	2b	5bc
ICP 8863	85c	0.66c	0b	0b	1c

[a] Similar alphabets did not vary significantly (p=0.05)

measured as AUDPC. Therefore, it is evident that most of the resistant genotypes have shown an equal capability for localization in roots with ICP8863 having maximum localization capability.

Components of quantitative resistance in sick plot experiment

Susceptible genotypes Bahar and TTB7 showed higher wilt incidence and thus higher AUDPC (Table 3). Incubation period (27–31 days) in susceptible genotypes was significantly shorter than the IP (54–76 days) noted in resistant genotypes; susceptible genotypes Bahar and TTB7 IP did not differ significantly. However, in resistant genotypes there was significant variation and ICP8863 had the longest period of 76 days. Similarly, a higher WI (4.3–4.6) in susceptible genotypes was significantly different from the lower WI (1.3–1.9) estimated in the resistant genotypes. Genotype ICP8863 had the minimum WI, 0.92. Overall, Bahar and TTB7 had the minimum IP and higher WI as expected, and resistant genotypes had highest IP and the lowest wilt index.

The number of colonized plants in susceptible pigeonpea genotypes was significantly higher than in resistant genotypes observed at 120 DAS. In susceptible genotypes, number of colonized plants was 32–36 which is much higher than resistant genotypes where the corresponding number was 5–17. In resistant genotypes, number of plants colonized by the pathogen showed significant variation and the number of plant colonized was lowest in genotypes ICP8863.

It appeared that there are quantitative differences among the resistant genotypes and ICP 8863 has maximum localization capability in root and stem.

Relationship between components of resistance and area under disease progress curve

AUDPC was significantly correlated with IP and number of plant colonized irrespective of root inoculation or sick plot experiment (Tables 4 and 5). IP and number of plants colonized was significantly correlated at 15, 30 and 45 DAI (Table 4) in the inoculation experiment and 120 DAS in the sick plot experiment (Table 5). Wilt index measured at 120

Table 3 AUDPC, wilt incidence and resistant components in sick plot infected pigeonpea plants

Genotypes	AUDPC	Wilt incidence % at 120 DAS	IP, days	WI at 120 DAS	Number of plants colonized at 120 DAS
Bahar	3909.0	62.3a[a]	27a	4.30a	32a
TTB7	3580.5	51.4a	31a	4.60a	36a
ICP7035	1476.7	14.5b	56b	1.90b	17b
ICP 9174	873.0	11.6b	65b	1.36b	12b
ICP 87119	405.0	12.4b	54b	1.50b	9b
ICP 8858	570.0	8.4b	61b	1.53b	12b
ICP 8863	355.0	5.6c	76c	0.92c	5c

[a] Similar alphabets did not vary significantly (p=0.05)

Table 4 Correlation coefficients between AUDPC and the components of wilt resistance incubation period (IP), weighted wilt index (WI) and number of plants colonized at 15, 30 and 45 DAI in pigeonpea inoculated with *F. udum*

		IP	WI at 120 DAS	Number of plants colonized at DAI		
				15	30	45
AUDPC		−0.8907*	0.6082	0.9764**	0.8202*	0.96484**
IP			−0.7040	−0.8572*	−0.7968*	−0.9138*
WI at 90 DAI				0.5156	0.5205	0.5920
Number of plants colonized at DAI	15				0.8075*	0.9331**
	30					0.7952*

*$P<0.05$; **$P<0.01$

DAS did not show any correlation between the components but could discriminate level of partial resistance between the resistant genotypes when number of plants colonized and IP did not differ significantly (Tables 4 and 5). Number of plants colonized at three different dates of observation had given significant correlations especially at 30 and 45 DAI (Table 4). Therefore, taking the number of plants colonized at 30 DAI and IP can be used to estimate AUDPC or resistance index. The relationship between AUDPC and the resistance components was given by

$$\mathrm{AUDPC} = 4845.799 - 16.127(\mathrm{N}) - 139.302(\mathrm{IP})$$

which explained the statistical relationship between the two variables with a coefficient of determination (R^2) of 0.986. This relationship may be viewed as an estimated resistance index (RI) for each genotype.

This study has determined the components of partial resistance to the wilt pathogen *F. udum* in pigeonpea genotypes. Of the three major resistance components studied, number of plants colonized and IP were significantly correlated with resistance of adult plants in field (AUDPC). A quantitative relationship established between two of the components, number of plants colonized, and IP and the AUDPC of genotypes grown in the field had a very high coefficient of determination (R^2=0.986). This suggests that a similar regression model may be useful in predicting the level of resistance in the field. Relationships between the slow rusting components and partial resistance in the field have been also reported in barley (Johnson and Wilcoxson 1978), downy mildew in lettuce (Eenink et al. 1982, Eenink and Dejong 1982) and anthracnose in *Stylosanthes hamata* (Iamsupasit et al. 1993). Although wilt index was not significantly correlated with AUDPC or any other component, it was useful in discriminating between genotypes that had similar incubation periods and numbers of colonized plants. Major components of partial resistance seemed to be those that restrict the growth of pathogen on or within host tissues (Lancashire and Jones 1985). Localization of the wilt pathogen (*F. oxysporum* s. sp *dianthi*) in carnation was ascribed to occlusion of xylem vessels, gel formation and phytoalexins accumulation (Baayen and van der Plas 1992), and regeneration of vascular tissues (Baayen 2005), which directly or indirectly restrict pathogen growth, and the property varied by cultivar. In pigeonpea, it appears that wilt resistance is attributed to slow growth and localization of the pathogen. Marley and Hillocks (1993) reported that at least part of the resistance in pigeonpea is derived from increased accumulation of phytoalexins. However, the role of phytoalexin accumulation in resistance has been negated as it could not explain resistance in ICCP8863 (Marley and Hillocks, 1993, 2002). Chaudhary and Kumar (2000) ascribed resistance to localization of pathogen due to narrower xylem vessels and vascular

Table 5 Correlation coefficients between AUDPC and resistance components in sick plot infected pigeonpea plants

	IP, days	WI at 120 DAS	Number of plants colonized at 120 DAS
AUDPC	−0.9282**	−0.3307	0.9791**
IP, days		0.0120	−0.9849**
WI at 120 DS			−0.4223

**$P<0.01$

bundle in roots in resistant cultivars. In the present study, in ICP8863 the high level of resistance is characterized by a long incubation period, maximum localization and low wilt index as compared with other resistant genotypes. These results suggest that the mechanism of resistance may be different in ICP8863. Confirmation of these apparent differences could have important implications for improvement of disease resistance. Reddy and Raju (1996) studied role of infection and colonization of four pigeonpea cultivars by an *F. udum* isolate and found that, although the cultivars differed in the extent of wilting, they did not differ in the extent of infection or colonization. Saxena and Khare (1988) have reported that lentil genotypes expressing lesser wilt had significantly shorter roots or fewer secondary roots. However, in pigeonpea the number of lateral or secondary roots has not been found to significantly correlate with wilt resistance (Prava 2007). The infection process in pigeonpea is not known. However, it is thought that penetration occurs through injured roots or root hairs or soft growing root tips. Considering that localization ability is mainly responsible for resistance then variation between genotype resistance index at 30 or 45 DAI using number of plants colonized could save time in determining resistance for quick selection in breeding programmes provided a sufficient number of plants is taken into consideration. Results obtained in this experiment indicated that resistant genotypes would be expected to delay the progress of wilt epidemic due to reduced root colonization as well as slow growth of the pathogen in xylem vessels. Provided resistance components show high heritabilities, selection among genotypes could be performed initially on the basis of number of plants colonized and IP.

Index of resistance estimated from multiple regression analysis of AUDPC on number of plants and IP may be useful as a tentative pre-field evaluation method to identify resistant genotypes. Griffiths and Jones (1987) used a similar strategy, applying multiple regressions to obtain an index that reflected disease ratings for cultivars under field conditions. Information on the components contributing to resistance could be easily obtained, compared with screening for disease resistance in the field.

A recommendation of different mechanisms through breeding could increase the level of resistance if the mechanisms are controlled by independent genes (Lancashire and Jones 1985). Further work on the expression components of resistance with apparently different resistance mechanisms and on the genetics and inheritance of individual components may contribute to the development of pigeonpea cultivars with increased levels of partial resistance.

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